

**The long polar fimbriae (*lpf*) operon and its flanking regions in bovine**

***Escherichia coli* O157:H43 and STEC O136:H12 strains**

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## ABSTRACT

Long polar fimbriae (Lpf) are intestinal adhesins and important virulence factors of pathogenic *Escherichia coli* strains. We cloned and sequenced the *lpf2-1* operon (*lpf2ABCD*) and its flanking regions of an intimin- and Shiga toxin-negative *E. coli* O157:H43 strain from bovine origin, and also sequenced the *lpf2-1* operon of 6 additional atypical O157 bovine *Escherichia coli* strains of various serotypes. Nucleotide sequence comparison of these *lpf* operons showed sequence conservation as they contain only four polymorphic nucleotide positions. Investigation of these O157 strains as well as 13 *Escherichia coli* Reference Collection (ECOR) strains carrying the *lpf2-1* allele revealed high degree of sequence conservation in the *lpf2* flanking regions. The *lpf2-1* allele is also present in a bovine Shiga toxin-producing *E. coli* STEC O136:H12 strain and *in vitro* adherence assays revealed that the absence of *lpf2-1* in this strain did not affect its host cell-binding properties. Our data indicate that *lpf2* loci is highly conserved in *E. coli* isolates, but its role in adherence might be masked by other uncharacterized adhesins.

## INTRODUCTION

*Escherichia coli* is an important member of the commensal intestinal microflora in mammals, but there are a large number of isolates, which have acquired a variety of virulence factors and are capable of causing serious diseases in humans and animals, including those classified as enterohemorrhagic *E. coli* (EHEC; Kaper et al., 2004). The frequent emergence of new isolates with new combinations of virulence genes is exemplified by the appearance of the strain responsible for the recent outbreak of hemolytic uremic syndrome (HUS) in Germany (Mellmann et al., 2011), and underlines the importance of the various possible lateral gene transfer mechanisms in the spread of virulence genes. Typical EHEC O157:H7/NM strains carry *stx* genes encoding Shiga-toxin and also harbour a pathogenicity island (PAI) known as the locus of enterocyte effacement (LEE), encoding the intimin adhesin, among other virulence factors (Kaper et al., 2004).

In addition to several extensively studied virulence factors carried by pathogenic *E. coli*, there are several additional factors such as the long polar fimbriae (Lpf), which represent a relatively recently described adhesin and virulence determinant in EHEC (Doughty et al., 2002). The exact mechanism by which Lpf contributes to the virulence of each pathogenic *E. coli* strain is currently under investigation, but there is well-documented evidence that Lpf promote adhesion of EHEC strains to the intestinal epithelium (Jordan et al., 2004; Fitzhenry et al., 2006; Torres et al., 2008) as well as showing that Lpf interacts with extracellular matrix proteins (Farfan et al., 2011).

Initially, two genetic variants of Lpf (Lpf1 and Lpf2) were identified in *E. coli*, and with the availability of additional sequence data, more variants have been discovered, some of which show a degree of association with certain serotypes and/or pathogroups (Torres et al., 2009). All known *lpf1* and *lpf2* operons are encoded on genomic islands, termed O islands,

integrated in specific chromosomal locations (Fitzhenry et al., 2006; Mellmann et al., 2011). The Lpf variant encoded by the operon first named *lpfA<sub>O113</sub>* (Ideses et al., 2005) and later termed as allele 1 of *lpf2* (*lpf2-1*) (Torres et al., 2009) is the most prevalent genetic variant of Lpf according to our present knowledge, as it has been detected in several strains from various serotypes (Toma C, 2004; Toma et al., 2006; Torres et al., 2009; Galli et al., 2010; Monaghan et al., 2011). In comparison, the integration site of the *lpf2* operon is found between the genes coding for the L-glutamine:D-fructose-6-phosphate aminotransferase (*glmS*) and that of phosphate-binding periplasmic protein (*pstS*). Recently, seven *E. coli* strains of the O157 serogroup (including strain T22), isolated from healthy cattle and without key EHEC virulence factors were found to harbour the Lpf2 variant (Sváb & Tóth, 2012). Three isolates were from the serotype O157:H43 (Tóth et al., 2009), and some of these members have been the focus of a recent study dealing with the evolution of the O157 serogroup (Iguchi et al., 2011).

In the current study, we report the sequence of the *lpf2* operon and its flanking regions in strain T22, a non-sorbitol-fermenting (NSF) O157:H43 with an atypical pathotype (*stx*-, *eae*), monitor the presence of the operon and its flanking regions in a collection of *E. coli* strains of various serotypes carrying the *lpf2-1* allele, and investigate the possible function of Lpf2 in adherence *in vitro* of a STEC O136:H12, that does not possess intimin or other known adhesins found in bovine STEC strains.

## MATERIAL AND METHODS

**Bacterial strains.** *E. coli* strains used in this study are listed in Table 1. The ECOR strains were provided by Mónika Kerényi (Department of Medical Microbiology and Immunology, Medical School, University of Pécs, Hungary). Strains were grown in lysogeny broth (LB), as well as on LB and bromothymol-blue agar plates. For isolation of genomic and cosmid DNA, strains were grown in tryptic soy broth (TSB).

**Cosmid clone library construction.** Genomic DNA was isolated from strain T22 with the phenol-chlorophorm method (Sambrook et al., 1982) after growing overnight in TSB. The preparation of the cosmid clone library was performed with pWEB-TNC Cosmid Cloning Kit (Epicentre, Madison, WI, USA) according to the manufacturer's instructions, with the modification that instead of mechanical shearing, genomic DNA was subjected to a partial digestion with restriction endonuclease *Mbo*I (Fermentas, Vilnius, Lithuania). Altogether, 1000 transformant colonies were kept as cosmid library.

**PCR screening for the presence of *lpf2* flanking regions.** The cosmid library was screened by PCR for the presence of *lpf2*. The primers and annealing temperatures used in the reactions are listed in Table 2. The strains listed in Table 1 were screened for the presence of flanking regions.

**Reverse Transcriptase PCR for *lpfA* of *E. coli* T22.** RNA was isolated from cells of a 48 h culture of *E. coli* strain T22 with RNEasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, with the modification that cells were collected with

centrifugation at 13,000 xg for 1 minute. After discarding the supernatant, the bacterial pellet was processed according to the manufacturers' instructions. RNA samples were treated with Sigma DNase I Amplification Grade (Sigma-Aldrich, St. Louis, MO, USA). The DNase treated samples were used as template for reverse transcription with Fermentas Maxima Reverse Transcriptase (Fermentas, Vilnius, Lithuania) according to the manufacturer's protocol. The product of this reaction was used as template in a regular PCR with the primers defined in Table 2.

**Sequencing.** A cosmid carrying the whole *lpf* operon was identified. DNA was isolated with the Sigma GenElute BAC DNA kit (Sigma-Aldrich, St. Louis, MO, USA), and was sequenced at Baygen Institute (Szeged, Hungary) using the combination of Life Tech's SOLiD 4, IonTorrent sequencing and the dideoxynucleotide methods. The products of the RT-PCR were also sequenced with the dideoxynucleotide method. Nucleotide sequence analysis and searches for open reading frames (ORFs) and homologous DNA sequences in the EMBL and GenBank database libraries were performed with the tools available from the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)), with Vector NTI and the CLC Bio DNA Workbench.

**Construction of an *lpfA2-1* deletion mutant of STEC O136:H12.** To generate an *lpfA2-1* deletion mutant of strain 187/06 (22), the *lpfA2-1* gene was replaced by a gene encoding kanamycin resistance using the lambda red recombinase system (Datsenko & Wanner, 2000). The long oligonucleotide primers used for introducing the mutation were those described by (Doughty et al., 2002). Each primer included 20 bp of sequence homologous to the kanamycin resistance gene, and 40 bp of sequence homologous to regions flanking the *lpfA2-1* gene. The kanamycin resistance gene was amplified from pKD4 by PCR. The purified PCR

product (1 µg DNA) was electroporated into 187/06 (22) strain which had previously been transformed with the lambda red recombinase expression vector, pKM201. Following electroporation, transformants of 187/06 (22) were recovered at 30°C for 2 h in LB broth and plated onto LB agar with kanamycin for overnight growth at 37°C to induce the loss of pKM201. Kanamycin-resistant colonies were then confirmed by PCR for replacement of *lpfA2-1*. The *lpfA2-1* deletion mutant of 187/06 (22) was complemented in trans by introduction of the entire *lpf2* operon on pWSK:lpf (kindly provided by Dr. E. Hartland).

**Bacterial adhesion.** The potential adherence capacity of strain T22 was investigated on primary bovine kidney and testis cells, which were kindly provided by Emília Szállás (Veterinary Diagnostic Directorate, National Food Chain Safety Office, Budapest, Hungary). The cells were grown to semi-confluency at 37°C in 5% CO<sub>2</sub> in 24 well plates in Roswell Park Memorial Institute 1640 (RPMI 1640) medium without supplements. Prior to use, cells were washed once with PBS. Strain T22 was grown for 48 hours with shaking at 200 rpm, and the cell monolayers were incubated for 5 hours with ca. 10<sup>10</sup> bacteria per well. The infected monolayers were washed two times with PBS, fixed with methanol and stained with Giemsa reagent.

The ability of *E. coli* O136:H12 *lpfA2-1*<sup>+</sup> strain and its deletion mutant to adhere to Hep-2, Caco-2 and T84 cell lines was assessed as previously described (Doughty et al., 2002), with minor modifications. The cells were grown to semi-confluency at 37°C in 5% CO<sub>2</sub> in 24 well plates (Falcon<sup>TM</sup> BD) in Dulbecco's minimal essential medium (DMEM), DMEM/F12 (Gibco, Carlsbad, CA, USA) or MEM, depending of the cell line, with 10% or 20% (vol/vol) heat-inactivated fetal bovine serum, 2 mM L-glutamine, and 1% (vol/vol) of a mixture of antibiotics/antimycotics (Gibco). Before use, the cells were washed twice with phosphate-buffered saline (PBS, Gibco) and replenished with the corresponding medium with no

supplements, as it was observed previously that mannose could inhibit Lpf mediated adherence (Farfan et al., 2011). The strains were grown static in LB broth overnight at 37°C, tissue culture cells were incubated with ca.  $10^7$  bacteria per well for 3 h at 37°C and 5% CO<sub>2</sub>. To quantify adherence, the infected monolayers were washed two times with PBS, and the adherent bacteria were recovered with 200 µl of 0.1% Triton X-100 in PBS and plated on LB agar plates containing the proper antibiotic. Data were expressed as the percentage of the bacterial inoculum recovered from triplicate wells and are the mean of at least two separate experiments. Statistical difference was expressed as the *P* value determined by a *t* test analysis. The *in vitro* competition assays were performed as described above except that cells were inoculated with  $5 \times 10^6$  cells each of mutant and wild type bacteria (total number of bacteria/well  $10^7$  cells) and competition index (CI) was calculated.

**Nucleotide sequence accession number.** The sequence of the *E. coli* T22 *lpf2* operon and its neighbouring regions has been deposited in the GenBank database under accession number AHZD01000104. The sequences of the *lpf2* operons of *E. coli* O157 strains B47, B54, T16, T34, T49 and T50 (see Table 1) were deposited under accession numbers KC207119, KC207120, KC207121, KC207122, KC207123 and KC207124, respectively.



## RESULTS AND DISCUSSION

**Sequence characteristics of the *lpf2* operon in the atypical bovine O157 strains.** We cloned and sequenced a 15.3 kb region from the genome of *E. coli* O157:H43 strain T22, including the *lpf2* operon. The sequence is deposited in GenBank under the accession number AHZD01000104. The schematic representation of the sequenced region is shown in Figure 1. According to our knowledge, this is the first time that the allelic variant *lpf2-1* operon and its flanking regions were sequenced in a non-sorbitol fermenting (NSF) strain of the serotype O157:H43. The *lpf2-1* operon itself has been detected earlier by PCR in other *E. coli* strains (Torres et al., 2009; Farfan et al., 2011).

In the *lpf2-1* operons of T22 and six other atypical O157 strains, there are only 4 positions that show polymorphism. One of them is a synonymous point mutation in the gene *lpfC* of strain T49, the others produce amino acid switches in the respective genes. The *lpf2A* gene is uniformly conserved in the investigated strains, and this is also true for the majority of the strains with whole genomes available in GenBank, an exception is the *lpf2A* gene of strain 55989 (Table 3.), which contains an isoleucine instead of a leucine in position 116. The *lpf2B* has an alanine instead of serine in position 99 in all the atypical O157 strains as opposed to the strains from GenBank listed in Table 3.

The *lpf2C* gene proved to be uniform in all the sequenced strains with the exception of T49, which has a cysteine in position 809 instead of tryptophane – the second polymorphism within the *lpf* operons of the atypical O157 strains. The only strain with an amino acid switch in *lpf2C* available in GenBank is E24377A, which has a leucine instead of proline in position 122. In the case of *lpf2D* gene, strain T22 has serine instead of alanine in position 341, this switch is shared with strains SE11, 55989 and 11368, and is the third polymorphism within the sequenced atypical strains. The fourth polymorphism can be observed in strains T49 and

T50, which encode a leucine instead of a methionine in position 313. The nucleotide sequence comparison of the *lpf* genes of the atypical O157 bovine strains is shown in Figure S1 (Supporting Material).

The existence of these polymorphisms indicate a similar level of variation in the otherwise conserved *lpf2* sequences. However, currently there is no data available on the potential or actual effect of these differences on the expression and/or function of Lpf2. The GC content of the sequenced *lpf* operons was 44%, while that of the flanking regions in T22 was 52%, close to the average GC content in the *E. coli* genome (McLean et al., 1998). This fact, together with the generally conserved sequence of *lpf2* in strains of various sero- and pathotypes (Table 3) suggests that these *lpf* variants might be located in genomic islands.

**Dissemination and characteristics of the flanking regions of *lpf2*.** The results of the PCR scanning of previously characterized strains carrying allele *lpf2-1* are listed in Table 1. The fact that the *lpf2-1* operon is flanked by the same set of genes in the majority of the strains sequenced so far (Table 3) is further demonstrated by the results of our PCR scanning. This uniformity indicates that the site between the *pstS* and *glmS* genes served as an integration hotspot at some point during the evolution of these strains. The genetic analysis of this region was performed in an earlier study, in which the authors designed primers specific for the flanking regions, and investigated whether the site between *pstS* and *glmS* is intact or interrupted by the *lpf2* operon (Toma et al., 2006). It must be noted however, that in the case of prototypic enteroaggregative strain *E. coli* (EAEC) strain 042 (O44:H18), a Tn21 transposon sequence is inserted between *lpfA* and *glmS* genes. This transposon element contains among other features transposases and genes encoding antibiotic resistance (Chaudhuri et al., 2010). Interestingly, four out of five strains which have the closest homologues to the *lpf2-1* operon of strain T22, are commensal isolates (Table 3).

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229 **Expression of Lpf2.** The RT-PCR specific for the *lpfA* genes from T22 yielded positive  
230 results, confirming transcription of Lpf2 in 48-hour cultures. In an earlier study with EHEC  
231 strain EDL933, one of the authors of the current manuscript found that the H-NS protein has a  
232 silencer role, while the regulatory protein Ler acts as an anti-silencer during the expression of  
233 Lpf1 (Torres et al., 2008). Our findings, as well as the lack of LEE (which includes the *ler*  
234 gene) in strain T22 suggests a different regulatory mechanism controlling Lpf2 expression  
235 relative to Lpf1.

236

237 **Contribution of LpfA2-1 to adherence of STEC O136:H12 *in vitro*.** The wide distribution  
238 of this particular allele of Lpf in pathogenic *E. coli* strains, especially in LEE-negative strains  
239 (Doughty et al., 2002) underlines its potential role as an important adhesin. There is both *in*  
240 *vitro* (Doughty et al., 2002; Newton et al., 2004; Torres et al., 2008; Farfan et al., 2011) and *in*  
241 *vivo* (Jordan et al., 2004) experimental evidence that Lpf enhances the adherence of intimin-  
242 positive and negative strains. Construction of an *lpf2* mutant in strain T22 resulted more  
243 challenging than expected; therefore, we created an *lpf2-1* mutant in STEC O136:H12 strain  
244 187/06 (22), a bovine isolate that possesses the same *lpf2-1* allele as strain T22 (data not  
245 shown) and flanked with the same genes (Table 3). The role of Lpf2 in adherence of strain  
246 187/06 (22) was evaluated using different tissue cultured cells lines; however, no clear  
247 differences could be observed in the quantitative adherence assays between O136:H12 and its  
248 corresponding *lpfA2-1* mutant. The strain 187/06 (22) did not show a significant reduction in  
249 the adherence neither to Hep-2 ( $P=0.10$ ), nor to Caco-2 ( $P=0.42$ ) cell lines when was  
250 compared to its deletion mutant, but exhibited a significant reduction in adherence to T84 cell  
251 line ( $P<0.0002$ ) (Figure 2). However, when competition adhesion assays were performed  
252 using the wild type and its corresponding *lpfA2-1* deletion mutant, the mean CI ( $3.27 \pm 1.35$ )

was significantly greater than 1 ( $P=0.043$ ). This finding, where the mutant strain is adhering more than the wild type suggested that the strain lacking Lpf might produce additional adhesion factors that provided a subtle advantage in the *in vitro* adherence assay.

In the case of strain T22, no specific adhesion could be observed in bovine testis and kidney cell cultures (data not shown), which is in harmony with the finding that strains possessing Lpf2 are not defective in adhesion but further analysis is needed to define the role of this fimbriae or other adhesion factors in these subset of strains.

In summary, we cloned and sequenced for the first time the long polar fimbriae-encoding operon and its flanking regions in an atypical, NSF O157:H43 *E. coli* strain. The Lpf operon itself is nearly identical to those of several other pathogenic and non-pathogenic strains from various serotypes and pathogroups, and represents the genetic variant termed allele 1 of *lpf2*. The integration site of the operon also shows high similarity to that on the aforementioned strains. Characteristic flanking regions were also found in other O157 non H7 strains carrying the same operon. Given the experimental evidence for the role of Lpf in adherence of *E. coli* O157:H7 and other pathogenic *E. coli* strains, it is plausible to propose that the Lpf2-1 fimbriae are important factors mediating adherence of our bovine *E. coli* strains. However, we did not observe any difference in the adherence profile of STEC 187/06 (22) or STEC O136:H12 strains. Based on recent published report, we speculate that in absence of an adhesin such as Lpf, the strains synthesized alternate surface structure as a compensatory mechanism for colonization (Lloyd et al., 2012). Further, the complete regulatory mechanisms of *lpf2* still need to be fully elucidated. Finally, the highly conserved sequence and integration site of the *lpf2* operon suggests that *lpf2* loci belongs to a conserved genomic island, and an interesting future task will be to elucidate the mechanism of genetic acquisition of this operon in pathogenic and commensal *E. coli* strains.

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411

412 **Table 1. List of strains used in this study and results of the PCR scanning of the flanking regions of the *lpf2* operon.**

Strain	Serotype	Phylogenetic group	<i>bgIG-phoU</i>	<i>phoU-pstB</i>	<i>pstB-pstA</i>	<i>pstA-pstC</i>	<i>pstC-pstS</i>	<i>pstS-lpfD</i>	<i>lpfA-glmS</i>	<i>glmS-glmU</i>	<i>glmU-atpC</i>	Reference
T16	O157:H43	B1 <sup>c</sup>	+	+	+	+	+	+	+	+	+	(Tóth et al., 2009)
T22	O157:H43	B1 <sup>c</sup>	+	+	+	+	+	+	+	+	+	(Tóth et al., 2009)
T34	O157:H43	B1 <sup>c</sup>	+	+	+	+	+	+	+	+	+	(Tóth et al., 2009)
T49	O157(rough):H9	B1 <sup>c</sup>	+	+	+	+	+	+	+	+	+	(Tóth et al., 2009)
T50	O157(rough):H37r	B1 <sup>c</sup>	+	+	+	+	+	+	+	+	+	(Tóth et al., 2009)
B47	O157:NM	B1 <sup>c</sup>	+	+	+	+	+	+	+	+	+	(Tóth et al., 2009)
B54	O157(rough):H12	A <sup>c</sup>	- <sup>a</sup>	+	- <sup>a</sup>	+	+	+	+	+	+	(Tóth et al., 2009)
ECOR7	O85:HN	A	+	+	+	+	+	+	+	+	+	(Ochman & Selander, 1984)
ECOR23	O86:H43	A	+	+	- <sup>a</sup>	+	-	+	+	+	+	(Ochman & Selander, 1984)
ECOR26	O104:H21	B1	+	+	+	+	+	+	-	+	+	(Ochman & Selander, 1984)
ECOR30	O113:H21	B1	+	+	+	+	+	+	+	+	+	(Ochman & Selander, 1984)
ECOR32	O7:H21	B1	+	+	+	+	+	+	+	+	+	(Ochman & Selander, 1984)
ECOR33	O7:H21	B1	+	+	+	+	+	+	+	+	+	(Ochman & Selander, 1984)
ECOR34	O88:NM	B1	+	+	-	+	+	+	+	+	+	(Ochman & Selander, 1984)
ECOR36	O79:H25	D	+	+	+	+	+	+	+	+	+	(Ochman & Selander, 1984)
ECOR57	ON:NM	B2	- <sup>b</sup>	- <sup>b</sup>	+	+	- <sup>b</sup>	- <sup>b</sup>	- <sup>b</sup>	- <sup>b</sup>	- <sup>b</sup>	(Ochman & Selander, 1984)
ECOR58	O112:H8	B1	+	+	+	+	+	+	+	+	+	(Ochman & Selander, 1984)
ECOR67	O4:H43	B1	+	+	+	+	+	+	+	+	+	(Ochman & Selander, 1984)
ECOR69	ON:NM	B1	+	+	+	+	+	+	+	+	+	(Ochman & Selander, 1984)
ECOR72	O144:H8	B1	+	+	+	+	+	+	+	+	+	(Ochman & Selander, 1984)
187/06 (22)	O136:H12	B2	+	+	- <sup>a</sup>	+	+	+	+	+	+	this study
C600	K12	A	-	+	+	+	-	+	+	+	+	(Appleyard, 1954)

413

414 <sup>a</sup>These strains yielded consistently longer product with the given primer pair.

415 <sup>b</sup> Strain ECOR57 yielded unspecific or weak products in all these reactions.

416   <sup>c</sup> The phylogenetic group of these strains was determined in Sváb & Tóth, 2012.

417   Abbreviations:

418   HN: H antigen non-typeable

419   NM: non-motile

420   ON: O antigen non-typeable

421

422 **Table 2. Primers used for the amplification of regions flanking the *lpf2* operon.**

Primer	Sequence (5'→3')	Genes amplified	Genbank number	Position amplified	Annealing temperature (°C)	Reference
bgIGfw	CCCAAGCGCTCCTGCGCTAAA	<i>bgIG-phoU</i>	CU928160.2	3977599-3978492	60	this study
phoUrev phoUfw	TCTGGAGTCGCTGGGCCGTC CGATCACGCGCTTCGCCAGA	<i>phoU-pstB</i>	CU928160.2	3978707-3979162	59	this study this study
pstBrev pstBfw	GGTATCGCCATTCGCCCCGGA GGACGGCCCCGATAAACGCCG	<i>pstB-pstA</i>	CU928160.2	3979526-3979912	60	this study this study
pstArev pstAfw	CAGCCGATCGCCAACCTGCC AGCCAGAACAGGCCGAAGGC	<i>pstA-pstC</i>	CU928160.2	3980508-3980919	59	this study this study
pstCrev pstCfw	ATCGGCGGCATCATGCTGGG ACCGTAGATCGGCACCAGCG	<i>pstC-pstS</i>	CU928160.2	3981370-3981924	58	this study this study
pstSrev pstSfw	CCAGAAAGGCGAAGATGCATGGC CAGACAGCGGCGCGTCAGAG	<i>pstS-lpfD</i>	CU928160.2	3982472-3983233	58	this study this study
lpfDrev lpfAfw	TGCTACCGAACCCAATACGGACAA TGTCGACAATTTACCGACGAAGT G	<i>lpfA-glmS</i>	CU928160.2	3987849-3988769	58	this study this study
glmSrev glmSfw	GCTGCCGAGCCGTATTGAGCA CGTGTGTCGCCCAGCGAGTA	<i>glmS-glmU</i>	CU928160.2	3989854-3990519	58	this study this study
glmUrev glmUfw	GGCGATGCGGAAATTGGCGA AGCAGATCGCCGCCGTGAC	<i>glmU-atpC</i>	CU928160.2	3991436-3992119	59	this study this study
atpCrev lpfA F	ACGAAGCGCGAGCCATGGAA ACCGCTATCGATGCTGAAGG	<i>lpfB-lpfA</i>	AY057066	678-1349	63	this study (Ideses et al., 2005)

lpfB R	GCGCAACATCTTCGGGAATA					
lpfC F2	CGCCGGGTTAGAAATAGATA	<i>lpfD-lpfC</i>	AY057066	3658-4421	63	(Ideses et al., 2005)
lpfD R2	TGCCTGGTTTATTTTGTGACGTA					
lpfA_inside_fw <sup>a</sup>	TCGACAGTAAATTGTGAATC	Part of <i>lpfA</i>	AY057066	233-790	50	this study
lpfA_inside_rev <sup>a</sup>	GAAGCGTAATATTATAGGCG					

423

424 <sup>a</sup> Primers used in RT-PCR for the detection of *lpfA* expression.

425

426 **Table 3. *Escherichia coli* strains with whole genomes in GenBank containing continuous *lpf2* operons with high homology to the *lpf2-1* of**  
427 **strain T22.**

Strain	Serotype	Pathotype	SNPs in the Lpf operon	Genbank number	Reference
SE11	O111:NM	commensal	6	AP009240.1	(Oshima et al., 2008)
11128		EHEC	7	AP010960.1	(Ogura et al., 2009)
55989		EAEC	8	CU928145.2	(Touchon et al., 2009)
KO11		commensal	9	CP002516.1	unpublished, JGI Project ID: 4085738
W	O26:H11	commensal	9	CP002185.1	(Archer et al., 2011)
11368		EHEC	9	AP010953.1	(Ogura et al., 2009)
IAII		commensal	10	CU928160.2	(Touchon et al., 2009)
E24377A		ETEC	11	CP000800.1	(Rasko et al., 2008)

428



429 **FIGURE LEGENDS**

430

431 **Figure 1. Schematic representation of the genetic region from *E. coli* strain T22 containing *lpf2*.** The relative length of the arrows is  
432 proportional to the relative length of the genes. Arrows representing genes from the same functional cluster are filled with the same pattern. The  
433 *pst* cluster encodes a phosphate ABC transporter, *phoU* encodes a phosphate transport regulator, and the product of *bgIG* is a putative  
434 transcriptional regulator. The *glm* cluster encodes an N-acetyl glucosamine-1-phosphate uridylyltransferase, and *atpC* encodes the epsilon subunit  
435 of ATP synthase.

436

437 **Figure 2. Comparative chart of *in vitro* bacterial adhesion assays to Caco-2, Hep-2 and T84 cell lines.** The results are expressed as the  
438 percentage of cell-associated bacteria from the original inoculum [(final CFU/ml / initial CFU/ml) x 100] and are the means  $\pm$  the standard error  
439 of at least two independent experiments in triplicate wells.

440

441

442 **Figure S1. Sequence comparison of the *lpf2* operons of atypical bovine *Escherichia coli* O157 strains.** The yellow arrows indicate the ORFs  
443 of the individual *lpf* genes. Point mutations are marked in red. The sequences of the *lpf2* operons were deposited under the following accession  
444 numbers: *E. coli* B47 (O157:NM) **KC207119**, *E. coli* B54 (O157:H12) **KC207120**, *E. coli* T16 (O157:H43) **KC207121**, *E. coli* T34 (O157:H9)  
445 **KC207122**, *E. coli* T49 (O157:H37) **KC207123**, *E. coli* T50 (O157:H43) **KC207124**

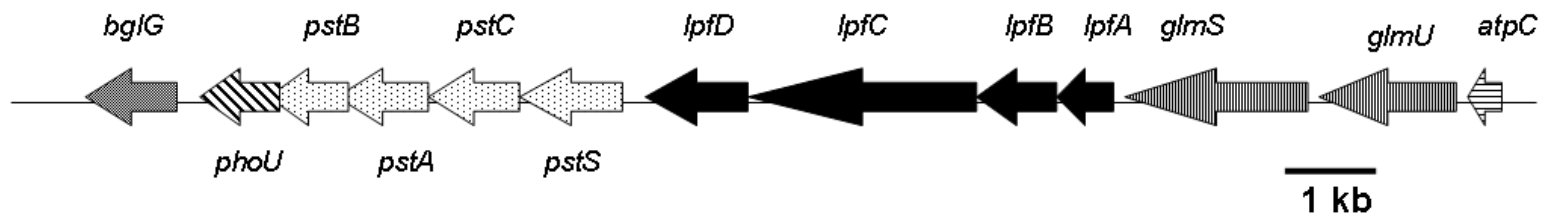
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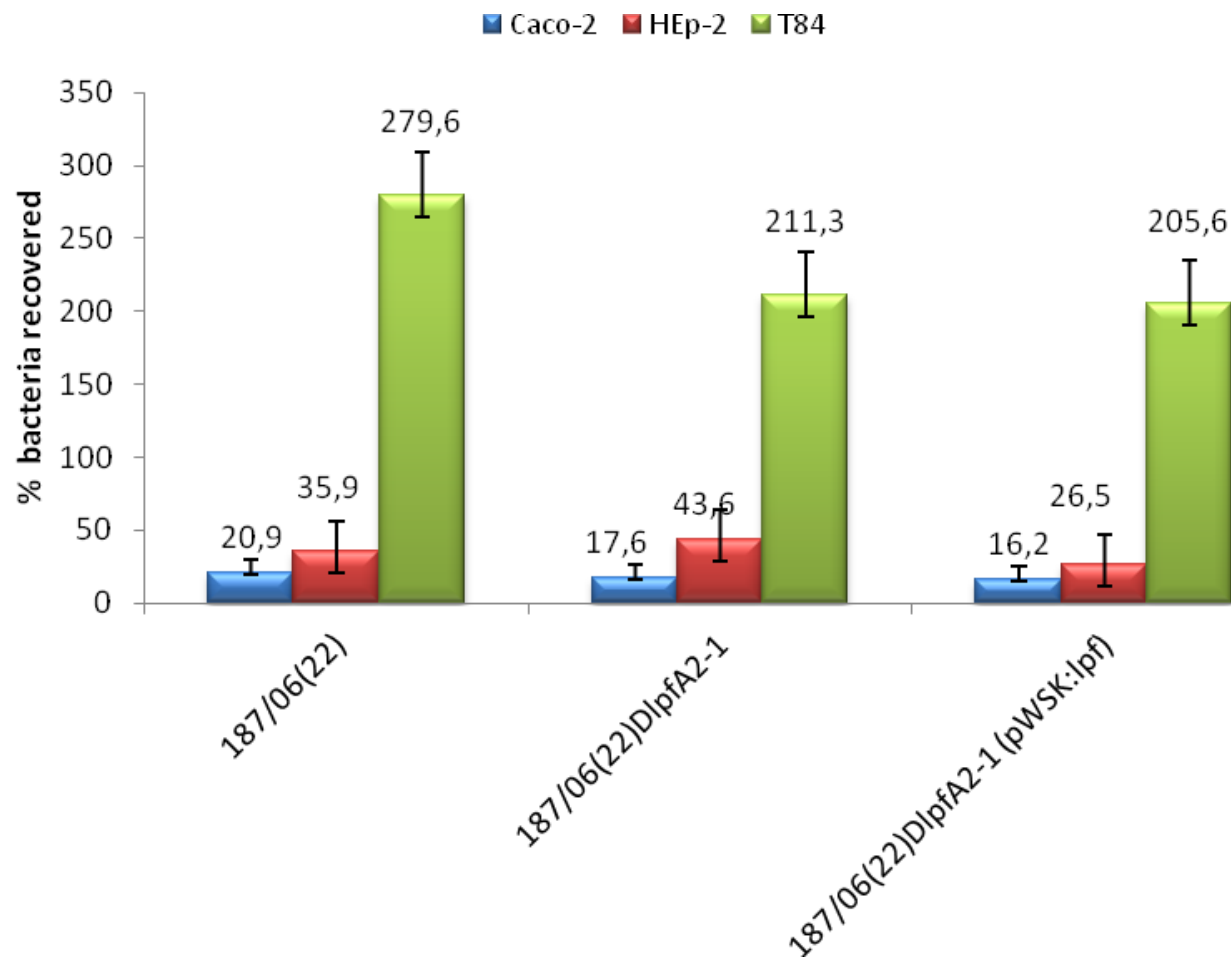
448 **FIGURES**

449

450 **Figure 1. Schematic representation of the genetic region from *E. coli* strain T22 containing *lpf2*.**



452 **Figure 2. Comparative chart of *in vitro* bacterial adhesion assays to Caco-2, Hep-2 and T84 cell lines.**



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